Attorney Docket No. C1041/7010 (AWS)

Appendix to Preliminary Amendment

Marked Up Version of Replaced Paragraphs

March 2, 2001

Example 14: Sequence specificity of the G-motif binding protein.

Extracts of J774 cells were incubated with single-stranded, radiolabeled GR1 for 30 min at room temperature. Different G-motif and non-G-motif containing competitors were added in a 150-fold molar excess. Treatment with Proteinase K or RNAse_A was performed before the binding reaction was initiated. Free probe was separated from complexes formed by running a 5 % non-denaturing PAGE.

Sequences of competitors:

G-motif containing ODN

GR1	TTGGA**GGGGG**TG**GTGGG**G	SEQ ID NO:17
EGR1	AGC**GGGGG**CGAGC**GGGGG**CG	SEQ ID NO 18
SP1	TCGAT**CGGGG**CG**GGGCG**AGC	SEQ ID NO 19

Non-G-motif containing ODN

Purα	AAAAGGGAAGGGATGGCT	SEQ ID NO 38
PurαOri	GGAGGCGGAGGCGGAGGCGGAGGC	SEQ ID NO 39
1668	TCCATGACGTTCCTGATGCT	SEQ ID NO 40
NFkB	ATATAAGGGAAATTTCCAGC	SEQ ID NO 41
GR1comp	CCCCACCACCCCCTCCAA	SEQ ID NO 42

As can be seen in Figure 14, a characteristic double banding pattern can be detected which is specific for G-motif ODN. Although Purα, PurαOri and NFkB ODN have very close approximations to the G-motif neither are capable of blocking the labeled G-motif ODN from binding its target. The target is a protein or protein as demonstrated by its loss upon [p]Proteinase K treatment. However RNAse A failed to destroy the target, thus the target is not RNA as would be the case for antisense ODN technologies.

Table 1: Prototypic blocking oligonucleotides

PZ1	5'	CTCCTAGCGGGGGCGTCCTAT	3'	SEQ ID NO:1
PZ2	5'	CTCCTAG**T**GGGGG**T**GTCCTAT	3'	SEQ ID NO:2
PZ3	5'	CTCCTA**TT**GGGGG**TT**TCCTAT	3'	SEQ ID NO:3
PZ4	5'	CTCCTA**GT**GG**TT**G**TG**TCCTAT	3'	SEQ ID NO:20
PZ5	5'	CTCCTA**GTT**G**TTTTG**TCCTAT	3'	SEQ ID NO:21
Poly-G	5'	**GGGGGGGG**GGGGG**GGGGGGGG**	3'	SEQ ID NO:22

Bold lettering represents changed nucleotides from the initial PZ1 ODN.

Table 2A: Data arranged to easily show the iterative process. Next to l[L]ast column of values from preliminary experiment.

				IC_{50} (nM)	IC_{50} (nM)	SEQ ID NO:
PZ31 (PZ3)	5'	CTCCTATTGGGGTTTCCTAT	3'	80 3	58.7	3
PZ32	5'	CTCCTATTGGGGTTTCCTAT	3'	187 2	182.0	4
PZ33	5'	CTCCTATTGGGTGTTTCCTAT	3'	516 0	138.5	5
PZ34	5'	CTCCTATTGGTGGTTTCCTAT	3'	1382 1	495.4	23
PZ35	5'	CTCCTATTGTGGGTTTCCTAT	3'	97 3	67.6	6
PZ36	5'	CTCCTATTTGGGGTTTCCTAT	3'	116 8	32.2	7
PZ37	5'	CTCCTATTGGGTTTTTCCTAT	3'	647 0		24
PZ38	5'	CTCCTATTGGTGTTTTCCTAT	3'	1003 0		25
PZ39	5'	CTCCTATTGTGGTTTTCCTAT	3'	916 0		26
PZ310	5'	CTCCTATTTGGGTTTTCCTAT	3'	344 6		27
PZ311	5'	CTCCTATTGGTTGTTTCCTAT	3'	1092 9		28
PZ312	5'	CTCCTATTGTGTGTTTCCTAT	3'	1392 1		29
PZ313	5'	CTCCTATTTGGTGTTTCCTAT	3'	985.4		30
PZ314	5'	CTCCTATTGTTGGTTTCCTAT	3'	2075.6		31
PZ315	5'	CTCCTATTTGTGGTTTCCTAT	3'	2230.3		32
PZ316	5'	CTCCTATTTTGGGTTTCCTAT	3'	684.0		33
PZ332	5'	CTCCTATTTTTTTTTTCCTAT	3'	>5000.0		34

Table 2B: Data arranged to easily show the affinity rank order of the oligonucleotides of the present invention. Range of affinity differences from highest to[o] lowest is greater than 62 fold. These data combined with the uptake data (Fig. 3) demonstrate that a sequence selective receptor on the surface of cells is responsible for DNA uptake. Because the receptor is sequence selective, high affinity interacting oligonucleotides can be designed which interfere with the potential inflammatory effects of CpG-motif containing DNA.

				IC_{50} (nM)	SEQ ID NO:
PZ31 (PZ3)	5'	CTCCTATTGGGGTTTCCTAT	3'	80 3	3
PZ35	5'	CTCCTATTG**T**GGGTTTCCTAT	3'	97 3	6
PZ36	5'	CTCCTATT**T**GGGGTTTCCTAT	3'	116 8	7
PZ32	5'	CTCCTATTGGGG**T**TTTCCTAT	3'	187 2	4
PZ310	5'	CTCCTATT**T**GGG**T**TTTCCTAT	3'	344 6	27
PZ33	5'	CTCCTATTGGG**T**GTTTCCTAT	3'	516 0	5
PZ37	5'	CTCCTATTGGG**TT**TTTCCTAT	3'	647 0	24
PZ316	5'	CTCCTATT**TT**GGGTTTCCTAT	3'	684 0	33
PZ39	5'	CTCCTATTG**T**GG**T**TTTCCTAT	3'	916 0	26
PZ313	5'	CTCCTATT**T**GG**T**GTTTCCTAT	3'	985 4	30
PZ38	5'	CTCCTATTGG**T**G**T**TTTCCTAT	3'	1003 0	25
PZ311	5'	CTCCTATTGG**TT**GTTTCCTAT	3'	1092 9	28
PZ34	5'	CTCCTATTGG**T**GGTTTCCTAT	3'	1382 1	23
PZ312	5'	CTCCTATTG**T**G**T**GTTTCCTAT	3'	1392.1	29
PZ314	5'	CTCCTATTG**TT**GGTTTCCTAT	3'	2075.6	31
PZ315	5'	CTCCTATT**T**G**T**GGTTTCCTAT	3'	2230.3	32
PZ332	5'	CTCCTATT**TTTTT**TTTCCTAT	3'	>5000.0[a]	34

a. greater than calculable range for assay

Table 3: Utilizing the motif GNGGG or GGGNG a determination of rank order of replacement nucleotides for N

				IC_{50} (nM)	SEQ ID NO:
PZ31 (PZ3)	5'	CTCCTATTGGGGGTTTCCTAT	3'	340.2	3
PZ35	5'	CTCCTATTG**T**GGGTTTCCTAT	3'	289.8	6
PZ35A	5'	CTCCTATTG**A**GGGTTTCCTAT	3'	247.0	8
PZ35C	5'	CTCCTATTG**C**GGGTTTCCTAT	3'	994.3	9
PZ33	5'	CTCCTATTGGG**T**GTTTCCTAT	3'	488.2	5
PZ33A	5'	CTCCTATTGGG**A**GTTTCCTAT	3'	649.0	10
PZ33C	5'	CTCCTATTGGG**C**GTTTCCTAT	3'	1122.5	35

Table 4: The effect of addition G flanking the motif lowers blocking affinity

				IC_{50} (nM)	SEQ ID NO:
PZ31 (PZ3)	5'	CTCCTATTGGGGGTTTCCTAT	3'	76.1	3
PZ31-G9	5'	CTCCTA**GG**GGGGG**GG**TCCTAT	3'	377.6	11
PZ31-G13	5'	CTCC**GGGG**GGGGG**GGGG**CTAT	3'	2050.1	36
PZ31-G17	5'	CT**GGGGGG**GGGGG**GGGGGG**AT	3'	3178.3	37
Poly G	5'	**GGGGGGGG**GGGGG**GGGGGGGG**	3'	1568.2	22

Table 5: The effect of random nucleotides flanking the motif increases blocking affinity. Blocking affinity was minimally [e]affected by position of the motif, however the G-motif at the 3' end had the greatest affinity.

				IC_{50} (nM)	SEQ ID NO:
PZ31 (PZ3)	5'	CTCCTATT**GGGGG**TTTCCTAT	3'	76.1	3
PZ31-Random	5'	HHHHHHHW**GGGGG**HHHHHHHH	3'	11.6	12
PZ31-Random-5'	5'	**GGGGG**HHHHHHHHHHHHHHHH	3'	<10.0	13
PZ31-Random-3'	5'	HHHHHHHHHHHHHHHW**GGGGG**	3'	<3.0	14

H= A, T, or C

W= A or T (W was used if preceding a G to avoid the CpG motif)

Table 6: Minimal length needed for high affinity block with G-motif flanked by random nucleotides

				IC_{50} (nM)	SEQ ID NO:
[PZ31 (PZ3)] PZ31-Random	5'	HHHHHHHW**GGGGG**HHHHHHHH	3'	[76.1] 11.6	12
PZ31-17	5'	HHHHHW**GGGGG**HHHHHH	3'	67.8	15
PZ31-13	5'	HHHW**GGGGG**HHHH	3'	570.0	16
PZ31-9	5'	HW**GGGGG**HH	3'	>3000.0	
PZ31-5	5'	**GGGGG**	3'	>3000.0	

H= A, T, or C

W= A or T (W was used if preceding a G to avoid the CpG motif)